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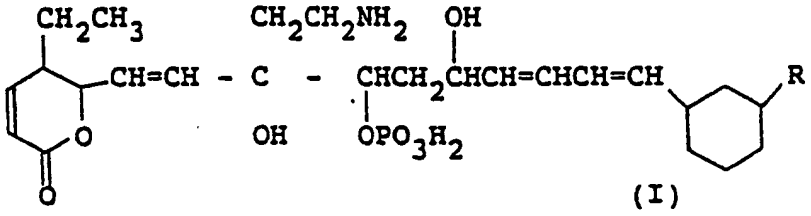
(54) 2-pyranone derivatives and process for production thereof.

(57) A 2-pyranone derivative represented by the formula (I):



(I)

wherein R represents a hydrogen atom, or a linear, branched alkylcarbonyloxy, or cyclo-alkylcarbonyloxy group having 3 to 10 carbon atoms, and salts thereof; a process for the production of the above-mentioned 2-pyranone derivative (I), comprising the steps of culturing a microorganism belonging to the genus Streptomyces and capable of producing the derivative, to produce the derivative, and recovering the produced derivative from the cultured product; a biocidal composition comprising the above-mentioned 2-pyranone derivative (I); and a microorganism belonging to the genus Streptomyces and capable of producing the above-mentioned 2-pyranone derivatives.

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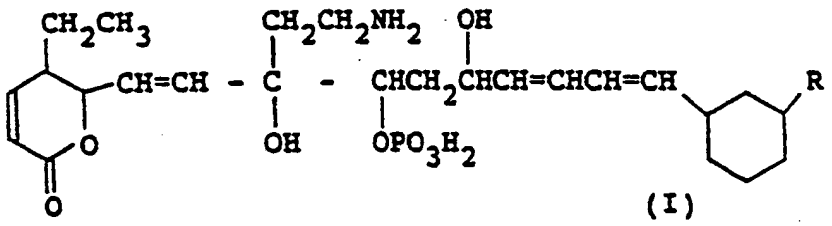
2-PYRANONE DERIVATIVES AND PROCESS FOR PRODUCTION THEREOF

The present invention relates to novel 2-pyranone derivatives, processes for production thereof, and to antimicrobial compositions containing them. In particular, the derivatives may exhibit antimicrobial activity at a very low concentration, especially against plant pathogenic fungi and therefore are useful as an active ingredient for a agricultural biocidal composition.

The problem addressed is the provision of new biocidal compounds which do not exhibit disadvantageous side effects on agricultural plants, and may be useful in particular for prevention and control of Botrytic cinerea which can be parasitic on a very wide range of plants (for example cucumber, tomato, eggplant, strawberry, lettuce, udo (Aralia cordata), and onion), at low temperature and high moisture condition.

In addressing this problem the present inventors worked to isolate from nature a microorganism which produces biologically active compounds suppressing gray mold, and found that an actinomyces belonging to the genus Streptomyces produces in a culture broth substances which exhibit a growth-preventing effect on fungi including dermatophytes and plant pathogenic fungi at a very low concentration. The present inventors further isolated and purified the substance and determined a structure thereof, and confirmed that the purified substance strongly suppresses gray mold.

Accordingly, the present invention provides a 2-pyranone derivative represented by the formula (I):



(I)

wherein R represents a hydrogen atom, or a linear or branched alkyl carbonyloxy, or cyclo-alkyl carbonyloxy group having 3 to 10 carbon atoms, and salts thereof.

In a second aspect there is provided a process for the production of a 2-pyranone derivative (I) as defined, comprising the steps of culturing a microorganism belonging to the genus Streptomyces and capable of producing the derivative, to produce the derivative, and recovering the produced derivative from the cultured product.

In a further aspect there is provided a biocidal composition comprising a biocidally effective amount of the above-mentioned 2-pyranone derivative. Still further, the present invention provides a microorganism belonging to the genus Streptomyces and capable of producing the above-mentioned 2-pyranone derivatives.

US-A-4578383, which corresponds to EP-A-87021, and EP-A-128651, describe a compound having a basic structure of the formula (I) above but having a hydrogen atom or a hydroxyl group at the 5-position of the pyranone ring, rather than the ethyl group in formula (I), and having a 1-propenyl or 3-hydroxy-1-propenyl at a position most distant from the pyranone ring, rather than a cyclohexyl ring as in formula (I). Therefore these compounds are significantly different in structure from the present compounds which have an ethyl group at the 5-position of the pyranone ring and a cyclohexyl ring structure at the position most distant from the pyranone structure. Moreover, the above-mentioned publications do not suggest that a microorganism belonging to Streptomyces might produce the compounds.

US-A-4575500 discloses a compound having a structure similar to that of formula (I) except for a cyclohexenone structure instead of the pyranone ring of the present invention.

Figure 1 represents an elution profile where components in a concentrated supernatant from a culture broth were separated by a high performance liquid chromatography.

Preferred features of the invention, and specific examples of embodiments, are now described.

In the above-mentioned formula (I), the alkylcarbonyloxy group is preferably butyryloxy, isobutyryloxy, isovaleryloxy, 2-methylbutyryloxy, cyclohexylcarbonyloxy, 4-methylhexanoyloxy, 6-methyl-heptanoyloxy, or octanoyloxy.

The producer microorganism may be any microorganism belonging to the genus Streptomyces and capable of producing the present 2-pyranone derivatives.

Such a microorganism may be obtained by isolating microorganisms belonging to Streptomyces from microbial sources such as soil, by a conventional procedure for the isolation of actinomyces, and selecting a microorganism which produces a substance having an antimicrobial activity against Botrytic cinerea. A producer strain embodying the invention thus obtained, Streptomyces platensis SAM-0654, was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology on January 22, 1988, as FERM BP-1668.

The strain SAM-0654 has the following taxonomical properties.

1. Morphology

An aerial mycelium shows simple branches, and at the top thereof, a spiral spore chain comprising 10 to 50 or more spores is formed. The spore is semi-spherical (crescent), has a length of 0.6 to 1.0 μm and a width of 0.3 to 0.4 μm, and has a smooth surface..

2. Cultural characteristics on various media

The appearance of a culture on various media is set forth in Table 1. The observation was carried out after culturing at 28° C for 21 days.

Table 1

Culture media	Growth	Aerial mycelium		Vegetative mycelium	Soluble pigment	Other appearance
Yeast extract - Malt extract Agar (ISP-2)	Good	Abundant	White - Light yellowish - Gray	Brown	Yellow	Hygroscopic
Oat meal agar (ISP-3)	Good	Abundant	White - Gray	Yellowish brown - Brown	Yellow	Hygroscopic
Inorganic salts - Starch agar (ISP-4)	Good	Abundant	White - Light yellowish - Gray	Yellow - Yellowish brown	Light yellow	Hygroscopic
Glycerol - Asparagine agar (ISP-5)	Good	Abundant	White - Light yellowish gray	Orange - Yellowish brown	Yellow	
1/10 V-8 agar	Good	Moderate	White - Gray	Deep greenish gray	-	Hygroscopic
Yeast extract - Starch agar	Good	Abundant	White	Yellow - Light yellowish brown	Light yellow	No spore formation
1/10 Potato - Carrot agar	Good	Moderate	White - Light gray	White - Light yellow	-	Hygroscopic
Calcium malate agar (+ Glycerol)	Good	Poor	White	Yellow	-	

3. Physiological properties

(1) Scope of growth temperature 20° C to 37°
(2) Liquification of gelatine negative
(3) Hydrolysis of starch positive
(4) Coagulation of skim milk (37° C) negative
(5) Peptonization of skim milk (37° C) negative

(6) Production of melanoid pigments

Tryptone-yeast extract agar medium negative

Tyrosine agar medium negative

Peptone-yeast extract-iron agar medium negative

(7) Nitrate reduction negative

(8) Utilization of carbon sources

L-arabinose	-	L-rhamnose	-
D-xylose	±	raffinose	+
D-glucose	+ +	D-mannitol	+ +
D-fructose	+	lactose	-
sucrose	+ +	salicin	-
inositol	+ +	cellulose	-

4. Chemotaxonomical properties

(1) 2,6-diaminopimelic acid

Whole-cell hydrolyzates of SAM 0654 contained L,L-2,6-diaminopimelic acid.

(2) Quinone system

The strain SAM 0654 has melaquinone-9(H6) and melaquinone -9(H8) as major quinones.

From the above-mentioned taxonomical properties, the present strain SAM-0654 is considered to be an actinomyces belonging to the genus Streptomyces. Comparison of the present strain with the known species of the genus Streptomyces, on the basis of the above-mentioned taxonomical properties, revealed that the present strain is most similar to Streptomyces platensis (International Journal of Systematic Bacteriology, Vol. 18, pp 360, 1968).

Accordingly, the present strain SAM-0654 was compared with a type strain of Streptomyces platensis, viz. Streptomyces platensis JCM 4662, and a summary of the results thereof is given in Table 2.

Table 2

			SAM0654	Streptomyces platensis JCM 4662
Spore chain			Spiral	Spiral
Surface and shape of spore			Smooth, subspherical (crescent)	Smooth, subspherical (crescent)
Cultural characteristics	Yeast extract - Malt extract agar (ISP-2)	Aerial mycelium	White - Gray	Gray
		Vegetative mycelium	Brown	Reddish brown
		Soluble pigments	Yellow	Light brown
		Other appearance	Hygroscopic	Hygroscopic
	Oat meal agar (ISP-3)	Aerial mycelium	White - Gray	Gray
		Vegetative mycelium	Yellowish brown	Reddish brown
		Soluble pigments	Yellow	Light brown
		Other appearance	Hygroscopic	Hygroscopic
	Inorganic salt - Starch agar (ISP-4)	Aerial mycelium	White - Gray	Gray
		Vegetative mycelium	Light yellowish brown	Reddish brown
		Soluble pigments	Light yellow	Light brown
		Other appearance	Hygroscopic	Hygroscopic
	Glycerol - asparagine agar (ISP-5)	Aerial mycelium	Light yellowish gray	Gray
		Vegetative mycelium	Yellowish brown	Yellowish brown
		Soluble pigments	Yellow	———
		Other appearance	———	———

Table 2 (continued)

	SAM0654	Streptomyces platensis JCM 4662
Production of melanoid pigments	negative	negative
Liquification of gelatine	negative	negative
Hydrolysis of starch	positive	positive
Coagulation and peptonization of skim milk	negative	negative
Utilization of carbon source	Glucose, fructose, sucrose, inositol, raffinose, mannitol	Glucose, fructose, sucrose, inositol, raffinose, mannitol

As seen from Table 2, properties of the SAM-0654 are very similar to those of Streptomyces platensis, and the only difference is that color tone of the vegetative mycelium and soluble pigments. In the type strain of Streptomyces platensis, Streptomyces platensis JCM 4662, the color tone of the vegetative mycelium and soluble pigments is reddish; on the other hand in the strain SAM-0654 it is yellowish. Nevertheless, the present inventors consider these differences to be not enough to separate the strain SAM-0654 from Streptomyces platensis, and then identified the strain SAM-0654 as Streptomyces platensis SAM 0654.

For a production of the present compound, a producer strain is preferably cultured in a liquid medium under an aerobic condition provided by shaking or by aeration and agitation, although a solid medium may be used. Any medium in which a producer microorganism can grow and produce the present compound

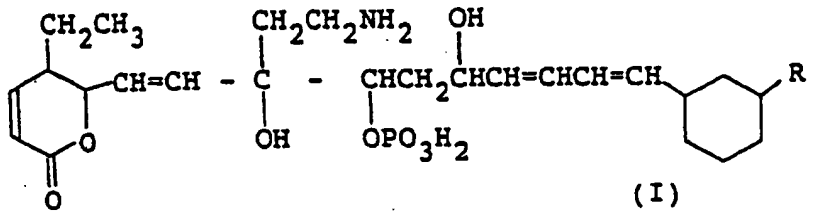
may be used, as long as the medium contains a carbon source such as glucose, lactose, glycerol, starch, sucrose, dextrin, molasses and/or organic acids, and a nitrogen source, such as hydrolyzed protein product such as pepton or casamino acid, meat extract, yeast extract, defatted soybean pellet, corn steep liquor, amino acids, ammonium salts, nitrate or other organic or inorganic nitrogen-containing substances. Inorganic salts, such as various phosphates, magnesium sulfate, and/or sodium chloride may be added to a medium. Moreover, vitamins and nucleic acid-related compounds may be added to accelerate the growth of the strain. Note, in some cases, the addition of an antifoamer such as silicone, propylene glycol derivatives, and soy bean oil may increase the accumulation level of the present compound.

When culturing, preferably a preculture is prepared on a small scale, and the preculture is innoculated to a production culture medium, although direct inoculation to a production culture medium may be allowed. Culture conditions including culture temperature, pH value of culture medium, and duration of culture are chosen or controlled so that the conditions provide a maximum production of the present compound. Usually, culturing is carried out at 25°C to 35°C, at pH 5.5 - 7.2, under an aerobic condition, for two to three days.

During the culturing, the present compounds are extracellularly accumulated in a culture broth. Therefore, in a preferred embodiment for recovering the products, a culture broth is filtrated or centrifuged to obtain a filtrate or supernatant containing the products, and the desired products are recovered from the filtrate or supernatant. Alternatively, the production can be directly recovered from a culture broth. The recovery process is followed by a disk method using Aspercillus oryzae or Botrytis cinerea as a test organism, or a cucumber seedling assay described in the examples.

The desired compound is isolated and purified from the culture broth by various procedures chosen according to the nature of the desired compound. Namely, extraction using a organic solvent, such as 1-butanol, which is immiscible with water and dissolves the desired compound, dissolution in a high polar solvent such as methanol or ethanol, removal of impurities by treatment with hexane or the like, gel filtration through Sephadex, ion exchange chromatography on an ion exchange resin, Sephadex ion exchanger or the like, or adsorption chromatography on active carbon, silica gel or Amberlite XAD-1 or -2, or a combination thereof, may be used to isolate and purify the desired compound. As especially preferable adsorbents, Diaion HP-20 (Mitsubishi Chemical Industry Ltd.), Sepabeads FP-DAI3 (Mitsubishi Chemical Industry Ltd.), YMC-C_{18} (Yamamura Chemical Laboratories Co. Ltd.), and a combination thereof, are mentioned. Note, since a culture broth from the producer strain contains more than ten analogues, separation and purification of each active component can be most effectively carried out by high performance liquid chromatography.

Figure 1 represents a chromatogram of separation of components shown by absorbance at 232 nm, obtained using a column YMC-C_{18} (50 mm of an inner diameter and 300 mm of a length, Yamamura Chemical Laboratories Co. Ltd.) and a gradient elution with 40% to 60% acetonitrile containing 0.1% formic acid at a flow rate of 32 ml/minute. Each peak was fractionated, the component was purified, and each component was found to have the following structure (I):



(I)

Referring to the above-mentioned general formula and Fig. 1;

Peak A in Fig. 1 represents a compound (I-a) embodying the invention, wherein R in the formula (I) is a hydrogen atom;

Peak B represents a mixture of a compound (I-b), wherein R is n-butyryloxy, and a compound (I-c), wherein R is isobutyryloxy;

Peak C represents a mixture of a compound (I-d), wherein R is isovaleryloxy, and a compound (I-e), wherein R is 2-methylbutyryloxy;

Peak E represents a compound (I-f), wherein R is cyclohexylcarbonyloxy;

Peak F represents a compound (I-g), wherein R is 4-methylhexanoyloxy;

Peak H represents a compound (I-h), wherein R is 6-methylheptanoyloxy; and

Peak I represents a mixture of a major compound (I-i), wherein R is octanoyloxy, and a compound (I-j), wherein R is $C_8H_{15}COO$-.

In addition to the above-mentioned compounds, it was found that minor compounds wherein R is, for example, propionyloxy, valeryloxy, or 4-methylvaleryloxy, are present, on the basis of NMR spectra and Mass spectra.

The present compounds can be present in the form of salts, such as hydrochloride, phosphate, nitrate.

The above-mentioned compounds embodying the invention suppress the gray mold at low concentration.

When the present compound is used as a anti-microbial agent, it may be admixed with a carrier, and if necessary, with other additives to form a conventional formulation used as an agricultural antimicrobial agent, such as solid, including finely divided powers and granules materials, as well as liquid, such as solutions, emulsions, suspensions, concentrates, slurries and the like. Suitable liquid carriers include water; alcohols such as ethanol, ethyleneglycol; ketones such as acetone; ethers such as dioxane, cellosolves; aliphatic hydrocarbons such as kerosene; aromatic hydrocarbons such as benzene, toluene; organic bases such as pyridine; halogenated hydrocarbons such as chloroform, carbon tetrachloride; esters such as ethyl acetate, fatty acid glycerol esters; nitriles such as acetonitrile; dimethylformamide; and dimethyl sulfoxide.

Suitable solid carriers include powders having a plant origin, such as starches, wheat flour; and mineral powders such as kaolin, bentonite, calcium phosphate, clays, talks, silicas. These can be used alone or in combination.

Moreover, as the emulsifier, fixing agent, penetrating agent or dispersant, soap, sulfate esters of higher alcohols, alkyl sulfonic acid, alkyl aryl sulfonic acids, tertiary ammonium salts, oxyalkylamines, fatty acid esters, polyalkyleneoxides, and anhydrosolbitols are used. Usually, these additives are used in an amount of 0.2 to 10% by weight relative to the formulation. Further, other antimicrobial agents, insecticides, nematocides, herbicides, plant growth regulators, plant nutrients, fertilizer, and/or soil modifiers can be included in the formulation.

Antimicrobial compositions of the present invention may be produced by conventional procedures from the 2-pyranone derivative (I), carriers, additives and the like.

The present compound (I) is preferably contained in an amount of 0.5 to 50% by weight in a solid formulation, and in an amount of 5 to 90% by weight in a liquid formulation. Note, the liquid formulations are preferably diluted by an appropriate amount of water, for example, 50 to 5000-fold, prior to application.

An amount of the present compound (I) used, and the ratio thereof to other components in the composition, depends on the stage of growth of a plant to which the composition is to be applied, condition of growth of the plant, a nature of the pathogen, condition of pathology, method of application and the like, and is usually 10 to 300 g of the present compound (I) per 10 ares. A concentration of the compound (I) in a liquid composition in use is usually 10 to 1000 ppm. The method of application of the composition is not particularly limited, and it can be applied by, for example, spraying, dusting or by irrigation, or in the form of a seed coat, as long as it is applied safely and effectively to a target plant.

Examples

The present invention will be further illustrated by, but is by no means limited to, the following examples.

Example 1. Production of compounds

First, 30 ℓ of a medium containing 90 g glucose, 300 g peptone, 600 g corn starch, 60 g yeast extract, 90 g dry yeast cells, and 30 g dipotasium phosphate (pH 7.0) was inoculated with a pure culture of Streptomyces platensis SAM-0654 (FERM BP-1668), and culturing was carried out in a small fermentor at 28 °C, with aeration at 30 ℓ/minute and agitation at 400 rpm, for 40 hours.

The culture broth thus obtained was centrifuged to obtain 27 ℓ of a supernatant, which was then passed through a column (14 cm x 33 cm) packed with Diaion HP-20 (Mitsubishi Chemical Industry Ltd.) to adsorb the product. The column was washed with 30 ℓ of water and 36 ℓ of 50% methanol, and was eluted with 18 ℓ of methanol to obtain a eluate having an anti-Aspergillus (or Botrytis) activity. The eluate was concentrated under a reduced pressure, centrifuged to eliminate impurities, and 400 ml of the concentrate was applied to a column (5.6 cm x 33 cm) packed with Sepabeads FP-DA13 (Mitsubishi Chemical Industry Ltd.), which had been washed with methanol, and the column was eluted with methanol. Since an anti-microbial activity was found at an elution volume of 3000 ml to 6600 ml, this fraction was concentrated under a reduced pressure, water was added to the concentrate, and the mixture was lyophilized to obtain 800 mg of

a dry preparation. Then 100 mg of the dry preparation was dissolved in methanol, and the solution was applied to a high performance liquid chromatography column (YMC C_{18} column, having an inner diameter of 50 mm and a length of 300 mm; Yamamura Chemical Laboratories Co. Ltd.). Elution was carried out with 40% acetonitrile in 0.1% formic acid for 8 minutes, a linear gradient of 40% to 60% acetonitrile in 0.1% formic acid for 60 minutes, and 60% acetonitrile in 0.1% formic acid for 10 minutes, at a flow rate of 32 ml/minute, while monitoring by absorbance of 232 nm, and 25.6 ml (0.8 minute) fractions were obtained. This procedure was repeated eight times.

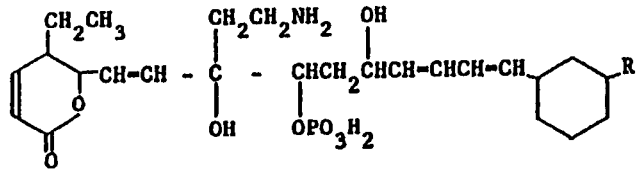
The chromatogram thereof is shown in Fig. 1.

Fractions No. 31 to 33 containing a peak A provided 13 mg of the above-mentioned compound I-a;
fractions No. 35 to 37 containing a peak B provided 50 mg of a mixture comprising the compound I-b and the compound I-c (1:2);
fractions No. 44.to 47 containing a peak C provided 120 mg of a mixture comprising the compound I-d and the compound I-e (3:2);
fractions No. 60 to 63 containing a peak E provided 119 mg of the compound I-f;
fractions No. 67 to 69 containing a peak F provided 35 mg of the compound I-g; and
fractions No. 81 to 87 containing peaks H and I provided 84 mg of a mixture comprising the compound I-h, the compound I-i, and the compound I-j (3:2:1).

Further, the mixture comprising the compounds I-a and I-c, the mixture comprising the compounds I-d and I-e, and the mixture comprising the compounds I-h, I-i, and I-j were separately subjected to high performance liquid chromatography under the same conditions as described above, and as a result, the compounds I-b and I-c, the compounds I-d and I-e, and the compounds I-h, I-i and I-j were isolated, respectively.

The compounds I-a to I-j thus obtained were white powders, exhibited a maximum ultraviolet absorbance at 232 nm in methanol, had a positive ninhydrin reaction, Ryndon Smith reaction, iodine reaction, anisaldehyde sulfuric acid reaction, and phosphomolybdic acidperchloric acid reaction, and a negative BTB and p-anisidine reaction. Note, as the compound I-j was obtained in only a small amount, the exact structure thereof was not determined. The structures and physico-chemical properties of the compounds I-a to I-i are shown in Table 3.

Table 3



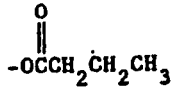
Compound	R	Molecular formula	Rf value*	IR spectrum (νcm^{-1})	NMR spectrum (in CD_3OD, δ ppm)
I-a	H	$C_{25}H_{40}O_8NP$	0.45	2968, 2934, 1730, 1604, 1137	7.09(1H,dd,J=10.0,5.0), 6.26(1H,m), 6.23 (1H,m), 6.08(1H,dd,J=15.5,6.5), 6.02(1H, dd,J=10.0,1.5), 5.92(1H,dd,J=15.5,1.0), 5.4(1H,m), 5.31(1H,m), 5.10(1H,ddd, J=6.5,5.5,1.0), 4.95(1H,m), 4.27(1H,ddd, J=10.0,10.0,3.0), 3.04(2H,m), 2.56(1H,m), 2.46(1H,m), 2.2(1H,ddd,J=7.0), 1.88(1H, ddd,J=7.0), 1.55-1.76(6H,m), 1.44-1.55 (2H,m), 1.24-1.42(3H,m), 1.04-1.24(3H,m), 0.95(3H,dd,J=7.5)
I-b		$C_{29}H_{46}O_{10}NP$	0.48	2968, 2935, 1728, 1603, 1093	0.93(3H,t,J=6.2), 0.94(3H,t,J=7.0), 1.04 (1H,m), 1.14(1H,m), 1.29(1H,m), 1.37-1.55 (3H,m), 1.55-1.67(4H,m), 1.72(1H,m), 1.78-1.89(2H,m), 1.89-2.01(2H,m), 2.20 (1H,m), 2.25(2H,t,J=7.2), 2.50-2.70(2H,m), 2.94-3.13(2H,m), 4.29(1H,dt,J=2.0,J=10.0), 4.70(1H,m), 4.95(1H,d,J=10.0), 5.10(1H,dd, J=5.0,J=6.8), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d,J=15.9), 6.02(1H,dd, J=10.0,J=1.5), 6.05(1H,dd, J=6.8,J=15.9), 6.22-6.33(2H,m), 7.08(1H,dd, J=5.5,J=10.2)

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Table 3 (continued)

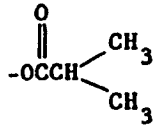
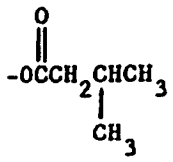
Compound	R	Molecular formula	Rf value*	IR spectrum (νcm^{-1})	NMR spectrum (in CD_3OD, δ ppm)
I-c		$C_{29}H_{46}O_{10}NP$	0.48	2968, 2935 1728, 1603, 1093	0.94(3H,t,J=7.0), 1.04(1H,m), 1.12(6H,d,J=7.0), 1.14(1H,m), 1.29(1H,m), 1.37-1.55 (3H,m), 1.55-1.67(2H,m), 1.72(1H,m), 1.78-1.89(2H,m), 1.89-2.01(2H,m), 2.20(1H,m), 2.49(1H,sep,J=7.0), 2.51-2.69(2H,m), 2.94-3.13(2H,m), 4.29(1H,dt,J=2.0,J=10.0), 4.70(1H,m), 4.95(1H,d,J=10.0), 5.10(1H,dd, J=5.0,J=6.8), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d,J=15.9), 6.02(1H,dd,J=10.0,J=1.5), 6.05(1H,dd,J=6.8,J=15.9), 6.22-6.33(2H,m), 7.08(1H,dd,J=5.5,J=10.2)
I-d		$C_{30}H_{48}O_{10}NP$	0.49	3374, 2872, 1726, 1383, 1294, 1188	0.94(6H,d,J=6.7), 0.95(3H,t,J=8.0), 1.05 (1H,m), 1.15(1H,q,J=11.8), 1.28(1H,m), 1.40-1.54(3H,m), 1.57-1.67(2H,m), 1.73 (1H,m) 1.78-1.89(2H,m), 1.91-2.01(2H,m), 2.04(1H,m), 2.15(2H,d,J=7.2), 2.19(1H,m), 2.56(1H,m), 2.63(1H,m), 3.00(1H,m), 3.08 (1H,m), 4.30(1H,dt,J=2.5,J=10.1), 4.73 (1H,m), 4.94(1H,m), 5.10(1H,dd,J=6.6, J=4.7), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d, J=15.6), 6.02(1H,dd,J=10.0,J=1.2), 6.05 (1H,dd,J=15.6,J=6.6), 6.24-6.32(2H,m), 7.08(1H,dd,J=10.0,J=5.0)

Table 3 (continued)

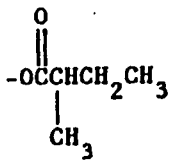
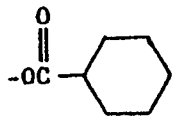
Compound	R	Molecular formula	Rf value*	IR spectrum (νcm^{-1})	NMR spectrum (in CD_3OD, δ ppm)
I-e		$C_{30}H_{48}O_{10}NP$	0.49	3374, 2872, 1726, 1383, 1294, 1188	0.89(3H,t,J=7.5), 0.95(3H,t,J=8.0), 1.05 (1H,m), 1.10(3H,d,J=6.9), 1.15(1H,q,J=11.8), 1.28(1H,m), 1.40-1.54(4H,m), 1.57-1.67(3H,m), 1.73(1H,m), 1.78-1.89(2H,m), 1.91-2.01(2H,m), 2.19(1H,m), 2.32(1H,m), 2.56(1H,m), 2.63(1H,m), 3.00(1H,m), 3.08 (1H,m), 4.30(1H,dt,J=2.5,J=10.1), 4.73(1H, m), 4.94(1H,m), 5.10(1H,dd,J=10.0,J=1.2), 6.05(1H,dd,J=15.6,J=6.6), 6.24-6.32(2H,m), 7.08(1H,dd,J=10.0,J=5.0)
I-f		$C_{32}H_{50}O_{10}NP$	0.50	3455, 2932, 1722, 1451, 1383, 1318, 1246, 1173	0.95(3H,t,J=7.5), 1.05(1H,m), 1.14(1H,q, J=11.8), 1.19-1.36(4H,m), 1.36-1.54(5H,m), 1.58-1.67(3H,m), 1.68-1.76(3H,m), 1.79-1.87(4H,m), 1.90-1.98(2H,m), 2.20(1H,m), 2.26(1H,m), 2.55(1H,m), 2.62(1H,m), 3.01 (1H,m), 3.07(1H,m), 4.29(1H,dt,J=2.4,J=10.1), 4.69(1H,m), 4.94(1H,m), 5.10(1H, dd,J=4.7,J=6.7), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d,J=15.6), 6.02(1H,dd,J=1.3,J=9.8), 6.06(1H,dd,J=6.7,J=15.6), 6.23-6.32 (2H,m), 7.08(1H,dd,J=5.1,J=9.8)

Table 3 (continued)

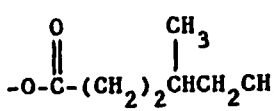
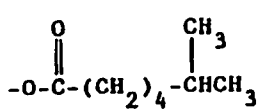
Compound	R	Molecular formula	Rf value*	IR spectrum (νcm^{-1})	NMR spectrum (in CD_3OD, δ ppm)
I-g		$C_{32}H_{52}O_{10}NP$	0.50	3358, 2963, 1726, 1711, 1464, 1450, 1383, 1253	0.84-0.91(6H,m), 0.95(3H,t,J=7.4), 0.98-1.23(3H,m), 1.23-1.55(7H,m), 1.55-1.77(4H,m), 1.77-1.89(2H,m), 1.89-2.02(2H,m), 2.15-2.35(3H,m), 2.51-2.68(2H,m), 2.95-3.13(2H,m), 4.28(1H,dt,J=2.0,J=10.0), 4.71(1H,m), 4.93(1H,m), 5.09(1H,dd,J=5.2,J=6.4), 5.31(1H,m), 5.45(1H,m), 5.94(1H,d,J=15.6), 6.01(1H,d,J=9.8), 6.05(1H,dd,J=6.4,J=15.6), 6.22-6.33(2H,m), 7.08(1H,dd,J=9.8,J=5.1)
I-h		$C_{33}H_{54}O_{10}NP$	0.52	2935, 2865, 1730, 1240, 1174, 1057	0.88(6H,d,J=6.7), 0.95(3H,t,J=7.5), 1.04(1H,m), 1.10-1.22(3H,m), 1.23-1.36(3H,m), 1.39-1.67(8H,m), 1.72(1H,t,J=12.2), 1.79-1.89(2H,m), 1.91-2.03(2H,m), 2.17-2.25(1H,m), 2.27(2H,t,J=7.4), 2.55(1H,m), 2.62(1H,m), 3.01(1H,m), 3.08(1H,m), 4.30(1H,t,J=9.4), 4.72(1H,m), 4.94(1H,m), 5.10(1H,m), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d,J=15.6), 6.02(1H,dd,J=9.8,J=1.2), 6.05(1H,dd,J=15.6,J=6.6), 6.23-6.32(2H,m), 7.08(1H,dd,J=9.8,J=5.1)

Table 3 (continued)

Compound	R	Molecular formula	Rf value*	IR spectrum (νcm^{-1})	NMR spectrum (in CD_3OD, δ ppm)
I-i	$-O-\overset{O}{\overset{\|\|}{C}}-(CH_2)_6CH_3$	$C_{33}H_{54}O_{10}NP$	0.52	2930, 2867, 1730, 1250, 1170, 1057	0.90(3H,t,J=7.0), 0.95(3H,t,J=7.5), 1.05 (1H,m), 1.15(1H,q,J=11.8), 1.19-1.37(8H, m), 1.38-1.54(4H,m), 1.54-1.76(5H,m), 1.79-1.89(2H,m), 1.91-2.01(2H,m), 2.17-2.25(1H,m), 2.27(2H,t,J=7.3), 2.55(1H,m), 2.62(1H,m), 3.01(1H,m), 3.08(1H,m), 4.30 (1H,dt,J=9.2,J=1.0), 4.72(1H,m), 4.94(1H, m), 5.10(1H,m), 5.31(1H,m), 5.46(1H,m), 5.95(1H,d,J=15.5), 6.02(1H,dd,J=9.8,J= 1.1), 6.05(1H,dd,J=15.5,J=6.6), 6.23-6.33 (2H,m), 7.08(1H,dd,J=9.8,J=5.1)

* Silica gel thin layer chromatography (Merck HPTLC Silica F254; developing solvent = chroloform/methanol/water 6:4:1; detected by bioautography using Aspergillus oryzae, anisaldehyde-sulfuric acid reaction and ninhydrin reaction.

Test for anti-fungal activity

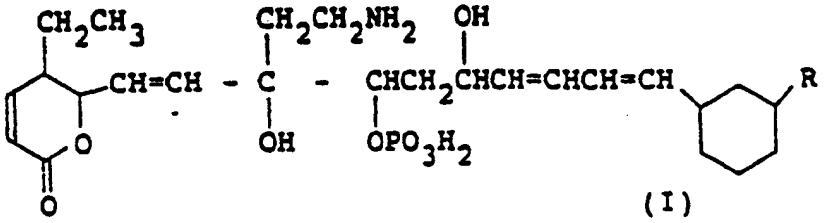
The disease suppressive activity of the present compounds I-a, I-c (and I-b), I-d (and I-e), I-f, I-g, I-h, and I-i against the gray mold was tested by the following procedure. A solution of the present compound having a predetermined concentration was coated,with an absorbent cotton block, on the cotyledon of a cucumber seedling seven days after seeding. One day after the coating, an agar disk having a diameter of 5 mm containing Botrytis cinerea was put on the coated cotyledon, the plant was incubated at 20 °C for three days, and the disease suppresive activity of the tested compound was observed. The results are shown in Table 4. In Table 4, Botrytis cinerea RR-4 is a strain resistant to antimicrobial agents (multi-resistant strain), isolated from a diseased seedling of egg plant, and Botrytis cinerea S-9 is a strain sensitive to antimicrobial agents, isolated from a diseased petal of orange blossom. Note, the extent of the disease suppressive effect is expressed by symbols + (no lesion), ± (lesion having a diameter of less than 10 mm formed), and - (lesion having a diameter of not less than 10 mm formed).

Table 4

Disease suppressive effect to Botrytis cinerea			
Test Compound	Concentration (ppm)	Botrytis cinerea S-9	Botrytis cinerea RR-4
I-a	125	+	+
	32	-	-
I-b and I-c (1:2)	125	+	+
	32	±	±
I-d and I-e (3:2)	125	+	+
	32	±	±
	8	-	-
I-f	32	+	+
	8	+	+
I-g	32	+	+
	8	+	+
I-h	32	+	+
	8	-	-
I-i	32	+	+
	8	-	-
Procymidone	125	+	-
	32	-	-
No treatment		-	-

Claims

1. A 2-pyranone derivative represented by the formula (I):



(I)

wherein R represents a hydrogen atom, or a linear or branched alkylcarbonyloxy, or cyclo-alkylcarbonyloxy group having 3 to 10 carbon atoms; or a salt thereof.

2. A 2-pyranone derivative according to claim 1, wherein R is butyryloxy, isobutyryloxy, isovaleryloxy, 2-methylbutyryloxy, cyclohexylcarbonyloxy, 4-methylhexanoyloxy, 6-methylheptanoyloxy, or octanoyloxy.

3. A process which comprises production of a compound according to claim 1.

4. A process for production of a 2-pyranone derivative (I) according to claim 1 or claim 2, comprising culturing a microorganism belonging to the genus Streptomyces and capable of producing the derivative, to produce the derivative, and recovering the produced derivative from the cultured product.

5. A process according to claim 4, wherein the microorganism is Streptomyces platensis SAM-0654 (FERM BP-1668).

6. A biocidal composition comprising an effective amount of a 2-pyranone derivative according to claim 1 or claim 2.

7. A microorganism belonging to the genus Streptomyces and capable of producing a 2-pyranone derivative according to claim 1 or claim 2.

8. A microorganism according to claim 7, which is Streptomyces platensis SAM-0654 (FERM BP-1668).

9. A method of treating plants to destroy pathogenic fungus, comprising applying to the plants a 2-pyranone derivative according to claim 1.

10. A method according to claim 9 wherein the pathogenic fungus is Botrytis cinerea.

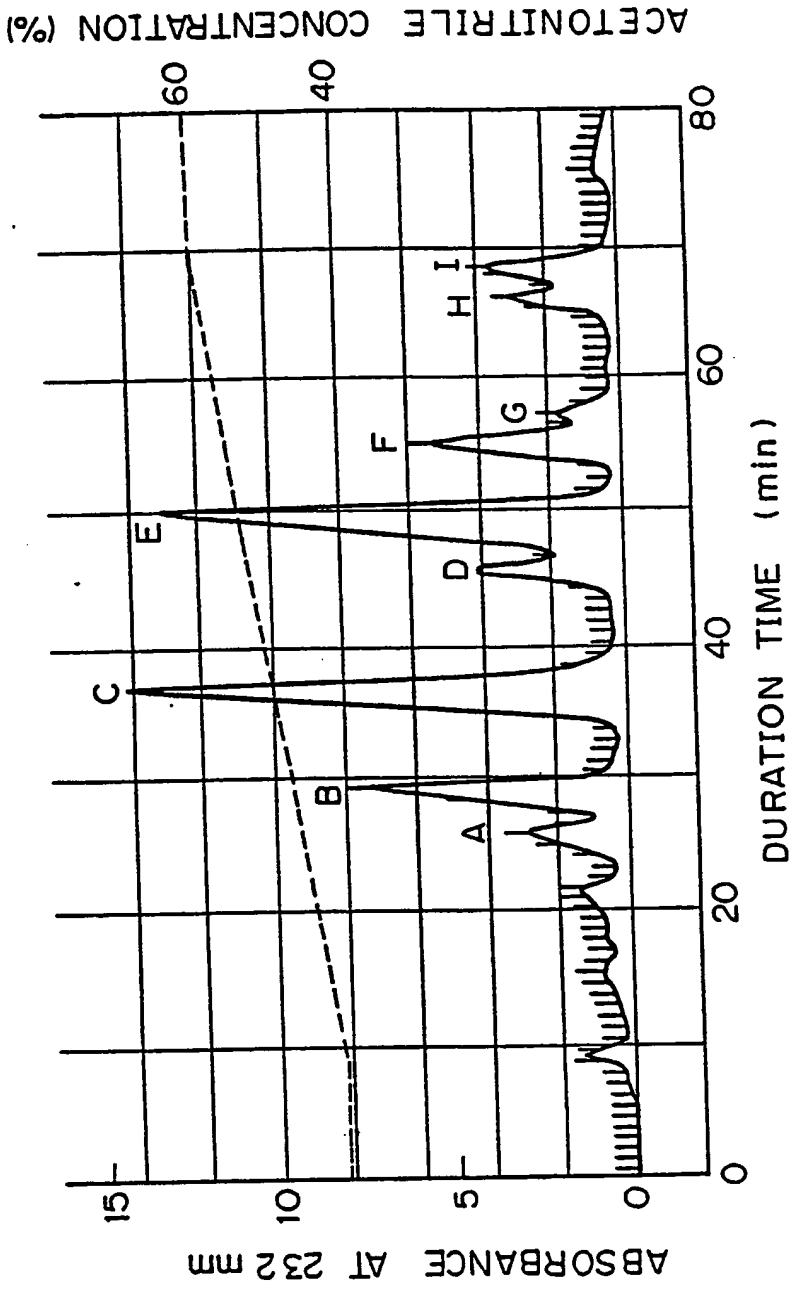

ACETONITRILE CONCENTRATION (%)
60
40
80
60
40
20
0
DURATION TIME (min)
ABSORBANCE AT 232 mm
15
10
5
0
A
B
C
D
E
F
G
H
I

Fig. 1